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Review

5-HT_{2A} receptors control body temperature in mice during LPS-induced inflammation via regulation of NO production

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ABSTRACT

G protein-coupled 5-HT_{2A} receptors are involved in the regulation of numerous normal and pathological physiological functions. At the same time, its involvement in the regulation of body temperature (T_b) in normal conditions is obscure. Here we study the effect of the 5-HT_{2A} receptor activation or blockade on T_b in sick animals. The experiments were carried out on adult C57BL/6 mouse males. Systemic inflammation and sickness were produced by lipopolysaccharide (LPS, 0.1 mg/kg, ip), while the 5-HT_{2A} receptor was stimulated or blocked through the administration of the receptor agonist DOI or antagonist ketanserin (1 mg/kg), respectively. LPS, DOI or ketanserin alone produced no effect on T_b. However, administration of LPS together with a peripheral or central ketanserin injection reduced T_b (32.2 °C). Ketanserin reversed the LPS-induced expression of inducible NO synthase in the brain. Consequently, an involvement of NO in the mechanism of the hypothermic effect of ketanserin in sick mice was hypothesized. Administration of LPS together with NO synthase inhibitor, L-nitro-arginine methyl ester (60 mg/kg, ip) resulted in deep (28.5 °C) and prolonged (8 h) hypothermia, while administration of L-nitro-arginine methyl ester alone produced no effect on T_b. Thus, 5-HT_{2A} receptors play a key role in T_b control in sick mice. Blockade of this GPCR produces hypothermia in mice with systemic inflammation via attenuation of LPS-induced NO production. These results indicate an unexpected role of 5-HT_{2A} receptors in inflammation and NO production and have a considerable biological impact on understanding the mechanism of animal adaptation to pathogens and parasites. Moreover, adverse side effects of 5-HT_{2A} receptor antagonists in patients with inflammation may be expected.

Contents

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1. Introduction

The involvement of the brain serotonin (5-HT) system in thermoregulation of homeotherms was already established in the early work of Feldberg and Myers [1]. The subsequently accumulated experimental data were a matter of great controversy. Apparently it is due to a whole set of 5-HT receptors coupled with different mechanisms of intracellular transduction which can produce similar or reversed functional effects [2]. The activation of 5-HT receptor subtypes such as 5-HT_{1A} [3,4], 5-HT_{1B} [5,6], 5-HT_{1D} [7] or 5-HT_3 [9,10] produces a reproducible but moderate hypothermic effect in rodents.

At the same time, the role of the 5-HT_{2A} receptor in thermoregulation is still obscure. In rats the activation of the 5-HT_{2A} receptor with its agonists MK-212, DOM or DOI increases the body temperature (T_b) by 1–2 °C [11–15] due to tail vasoconstriction i.e., reduced heat loss [16] and increase of O_2 consumption i.e., heat production [15,17]. However, 5-HT_{2A} receptors do not seem to be involved in the thermoregulation of mice: no effect of their pharmacological stimulation or blockade on T_b in mice was observed [18].

Here we hypothesized that the role of the 5-HT_{2A} receptor may be significantly increased in "extreme" conditions for example during inflammation. Administration of bacterial endotoxin lipopolysaccharide (LPS) produces sickness and systemic inflammation accompanied by enhanced blood levels of proinflammatory cytokines such as interleukin(IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α) [19] as well as fever in rats [20,21]. LPS also activates the expression of molecular mediators of inflammation such as Il-1β, IL-6, TNF-α and inducible NO synthase (NOS2) in the central nervous system [22–24].

The aim of our present study was to investigate the role of 5-HT_{2A} receptors in the regulation of T_b in sick mice. For this purpose, we intended (1) to compare the effects of the 5-HT_{2A} receptor agonist or antagonist on T_b in saline-treated (control) and LPS-treated (sick) mice; (2) to study the role of the central 5-HT_{2A} receptor in the regulation of T_b in LPS-treated mice; (3) to investigate the interaction between total metabolisms and T_b in sick mice; (4) to study the association between 5-HT_{2A} receptors, molecular mediators of inflammation such as IL-1β, IL-6, TNF-α, NOS2 and T_b.

2. Methods

2.1. Animals

This study was conducted in the Center for Genetic Resources of Laboratory Animals at the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (RFMEFI61914X0005 and RFMEFI62114X0010). Experiments were carried out on adult male mice of C57BL/6J strain. All animals were 10–12 weeks old and weighting 23.5 ± 2.0 g. After weaning, the mice were separated by sex and kept 6 per cage (29 × 18 × 16 cm) under standard conditions (ambient temperature 22–24 °C, natural light-dark cycle (14 h—light, 10 h—dark) and free access to water and food). Two days before experiments the mice were isolated into individual cages to remove the group effect. The experimental groups were composed of mice taken from different cages. All procedures were in compliance with the European Communities Council Directive of November 24, 1986(86/609/EEC) and approved by the ethics committees of Institute of Physiology and Fundamental Medicine and Institute of Cytology and Genetics SD RAS. All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs and treatments

Lipopolysaccharide (LPS, *Escherichia coli* 055:B5, Sigma–Aldrich Inc., USA) was diluted in sterile saline and injected ip at the dose of 0.1 mg/kg. Earlier we have shown that this dose of LPS was sufficient to produce such symptoms of sickness as locomotion deficit and catalepsy in C57BL/6 mice [25]. The 5-HT_{2A} antagonist -{2-[4-(4-fluorobenzoyl) piperidin-1-yl]ethyl}quinazoline-2,4(1H,3H)-dione (ketanserin, Sigma–Aldrich Inc., USA, 1 mg/kg), 5-HT_{2A} agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI, Sigma–Aldrich Inc., USA, 1 mg/kg) and NO synthase inhibitor *N*-nitro-L-arginine methyl ester (L-NAME, Sigma–Aldrich Inc., USA, 60 mg/kg) were diluted in sterile saline and injected ip two hours after LPS or saline injection. Earlier we have shown that these doses of ketanserin and DOI modified sickness symptoms in LPS-treated mice [26]. The dose of L-NAME was shown to be sufficient for NO synthase inhibition [27].

For the central administration, ketanserin was diluted in sterile saline and injected under short term light diethyl ether anesthesia (the time between onset and offset of the ether vapor was 40–50 c) at the dose of 40 nmol in 5 μl into the left lateral ventricle. The control animals were injected with sterile saline into the left lateral ventricle [10,28]. A 10 μl hamilton syringe was used for an ivc injection. The needle length was limited to 2.5 mm with a piece of 48.5 mm polypropylene tube (outer diameter 5 mm, inner diameter 0.9 mm). The injection accuracy was controlled by the needle trace position when the animals were decapitated and the cortex and hippocampus were dissected. In 82% of mice the needle hit the left ventricle. The mice with a verified ivc injection were taken for further statistical calculations.

2.3. Experiment design

Since effect of LPS was shown to be dependent on ambient temperature [29] and the mice were adapted to ambient temperature of 22–24 °C from birth, all our experiments were performed at 22.5–23.5 °C. The same group of thirty-six mice was used in the experiments 1 and 4, while two other groups of 24 and 31 mice were used in the experiments 2 and 3, respectively.

Experiment 1 was performed to compare the effects of the 5-HT_{2A} receptor agonist DOI or antagonist ketanserin on T_b in saline-treated (control) and LPS-treated (sick) mice. Thirty-six mice with implanted electronic thermometers were divided into six experimental groups of six and were treated ip with saline or LPS. Two hours later, the animals of six groups were treated